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**Viral mimic poly-(I:C) attenuates airway epithelial T cell suppressive capacity;
implications for asthma**

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Take home message: Airway epithelial regulation of T cell activity may be impaired during
virus-induced exacerbations in asthma.

Running title: Epithelial regulation of T cell activity

Key words: Airway epithelial cells; Allergic inflammation; Asthma immunology; T cell-
modulation

1 *To the Editor*

2 In allergen-sensitized asthmatic individuals, allergen-specific type-2 T-helper (Th2) cells
3 proliferate and secrete type-2 cytokines (e.g. interleukin (IL)-4, -5 and -13), driving the airway
4 inflammatory response that gives rise to the clinical symptoms of asthma. Both early-life
5 sensitization to aeroallergens and lower respiratory viral infections are important environmental
6 risk factors for developing asthma. Respiratory viral infections are also the most common trigger
7 for asthma exacerbations. Of interest, many asthma susceptibility genes are expressed in the
8 airway epithelium[1], which forms the first continuous line of defense against inhaled
9 environmental insults, including viruses and aeroallergens. Impaired immune regulation and
10 failure to maintain tolerance to allergens is thought to contribute to allergic sensitization. Asthma
11 epithelium may be deficient in its innate immune defense against virus infections, resulting in
12 increased viral replication upon rhinovirus infection compared to non-asthma-derived epithelial
13 cultures[2]. Furthermore, there is evidence for loss of the mucosal immune barrier in asthma,
14 with disruption of epithelial integrity[1,3]. This may not only lead to increased permeability, but
15 also result in the release of pro-inflammatory mediators, specifically of cytokines that drive type-
16 2 responses[3,4]. We recently observed that the ability of allergens to disrupt epithelial barrier
17 function is related to the development of type-2-mediated inflammation in asthma[5,6].
18 Furthermore, we demonstrated that healthy murine lung epithelium is a potent inhibitor of T cell
19 proliferation and that this inhibition is lost upon viral infection[7]. It is unknown if this immune
20 regulatory effect is displayed by human epithelium and is dysregulated in asthma. We
21 hypothesize that changes in this regulatory effect translate into aberrant regulation of T cell
22 responses in asthma. We studied the epithelial regulation of T cell proliferation and cytokine
23 responses upon epithelial stimulation with a viral mimic, using co-culture of human T cells and
24 primary bronchial epithelial cells (PBECS) from healthy controls and asthma patients.

1 Normal PBECs obtained from Lonza (Walkersville, MD) were used, except for the
 2 comparison between healthy and asthmatic epithelium, when PBECs derived by bronchial
 3 brushings from 5 healthy individuals and 5 patients with mild-moderate asthma, all non-smokers,
 4 were used (see fig. 1E for further characteristics). The study was approved by the Medical Ethics
 5 Committee of the UMCG. All subjects gave their written informed consent. PBECs were
 6 cultured in hormonally-supplemented bronchial epithelium growth medium (BEGM, Lonza) as
 7 described before[8-10]. PBECs were seeded in 12-well plates, grown to ~90% confluence,
 8 placed in BEGM/1% FCS overnight and pre-treated with/without 12.5µg/ml poly-(I:C) for 8
 9 hours and washed before placing in co-culture with T cells. CD4⁺ T cells were isolated from 10
 10 ml peripheral blood of healthy non-allergic non-smoking volunteers by Ficoll-Hypaque
 11 (Lymphoprep; Nycomed, Oslo, Norway) by density-gradient centrifugation followed by MACS
 12 sorting using CD4⁺ T cell Isolation Kit II (Miltenyi Biotec, San Diego, CA). T cells were seeded
 13 in duplicates at a concentration of 1.25*10⁵/ml in 12 well plates with/without PBECs (2:1
 14 PBECs:T cells) or in the upper well of a transwell system (0.4µM pores, Corning Costar) placed
 15 above PBECs. T cells were stimulated for 96 hours in the presence/absence of PBECs in
 16 BEGM/1% FCS or in conditioned-medium from 24 hours PBEC culture. T cell proliferation was
 17 induced by α-CD3/α-CD28 immobilized to beads (Dynabeads, Invitrogen, Carlsbad, CA) and
 18 analyzed by labeling with 10 µM carboxy fluorescein succinimidyl ester (CFSE, Life
 19 Technologies, Grand Island, NY) just prior to stimulation. CFSE dilution measurements were
 20 performed using flow cytometry (Calibur, BD) in viable CD4⁺/7-amino-actinomycin-D^{negative} T
 21 cells. The division index was calculated at (100-Y)/Y, where $Y(\%) = x_0 + x_1/2 + x_2/4 + x_3/8 +$
 22 $x_4/16$, x_0 = % of T cells that have not divided and x_{1-4} = T cells within progressive CFSE division
 23 gates. Levels of interferon (IFN)-γ, IL-4, IL-5, IL-10 and IL-13 were analyzed in cell-free

supernatants using a multiplex ELISA kit (Millipore, Billerica, MD) and Luminex technology (Luminex Corporation, Austin, TX).

We first studied the regulatory effects healthy human PBECs on T cell proliferative responses and observed an almost complete prevention of α -CD3/ α -CD28-induced T cell proliferation, with suppression of all T cells (fig. 1A, B), without significant effects on T cell viability (not shown). We previously observed in mice that T cell proliferation is equally well suppressed by lung epithelial cells, whether they were cultured submerged or at the air-liquid interface (data not shown). Here, we show that the suppressive effect was still present when PBECs and T cells were separated in a transwell system, suggesting the effect does not require direct cell-cell contact (fig. 1B). In addition, the suppressive effect could be transferred by conditioned-medium derived from 24-hours culture of the epithelial cells (fig. 1C), indicating the involvement of one or several soluble mediators. Assessing cytokine production, there was no significant effect on the type-1 cytokine IFN- γ , while healthy PBECs strongly inhibited the α -CD3/ α -CD28-induced secretion of the type-2 cytokines IL-4, IL-5, IL-10 and IL-13 (fig. 1D). PBECs cultured alone did not secrete detectable levels of any of these cytokines.

To study whether airway epithelium in asthma is deficient in its immunosuppressive capacity, we compared the effect of PBECs from asthma patients and healthy controls (fig. 1E). Both asthma-derived and control-derived PBECs formed confluent cell monolayer, without morphological differences as observed by microscopy, and both markedly inhibited T cell proliferation (fig. 1F). However, asthma-derived PBECs were significantly less potent in doing so (fig. 1F). Furthermore, PBECs from both asthmatics and healthy controls had a similar capacity to inhibit type-2 cytokine production, as shown for IL-13 (fig. 1G), without significant differences between the subject groups.

1 We previously showed that regulatory effect of healthy murine epithelial cells was lost
2 upon viral infection[7]. Respiratory RNA viruses act on Toll-like receptor (TLR)3, potentially
3 mediating airway inflammation in virus-induced asthma exacerbations[11,12]. Therefore, we
4 used the TLR3 agonist poly-(I:C) as a viral mimic. PBECs from controls and asthmatics were
5 exposed to poly-(I:C) and then washed, leaving the cells viable and fully confluent, before T
6 cells were added. Pre-treatment with poly-(I:C) strongly reduced the suppressive effect of both
7 control and asthma-derived PBECs on T cell proliferation (fig. 1H, 1I) and attenuated the
8 inhibitory effect of control PBECs on type-2 cytokine secretion, as shown for IL-13 (fig. 1J).

9 Together, our data suggest that asthma and viral infection compromise the
10 immunosuppressive capacity of airway epithelium, which may be most impaired during virus-
11 induced exacerbations of asthma, resulting in severe inflammation and a loss of asthma control.
12 Such a major compromise of epithelial immune suppressive capacity was not found and not
13 expected in the epithelium from stable asthma patients studied here. Viral infection of murine
14 pulmonary epithelial cells strongly reduces their T cell suppressive capacity[7] and our current
15 data indicate that viral infection of human airway epithelial cells will have a similar effect. Even
16 without using live virus, the epithelial immunosuppressive effect on T cells was strongly
17 attenuated upon exposure to the viral mimic and TLR3 agonist poly-(I:C). We speculate that
18 such inhibition of epithelial immune regulation could enhance type-2 cytokine secretion during
19 viral infection in early life, an important risk factor for asthma development, and during virus-
20 induced asthma exacerbations. Indeed, viral infection is accompanied by an increase in type-2
21 cytokine secretion in mouse models[13] and in asthmatic airways, where type-2 cytokine levels
22 relate to exacerbation severity[14].

1 In conclusion, we show that human bronchial epithelium exerts potent inhibitory effects
2 on T cell proliferation and type-2 cytokine secretion. These effects are attenuated following
3 exposure to a viral mimic, and asthma epithelium displays reduced inhibition of T cell
4 proliferation. Identification of the responsible mechanisms and mediator(s) involved in epithelial
5 immune regulation may provide new targets for novel disease modifying therapeutic and
6 preventive strategies in asthma.

References

1. Nawijn MC, Hackett TL, Postma DS, van Oosterhout AJ, Heijink IH. E-cadherin: gatekeeper of airway mucosa and allergic sensitization. *Trends Immunol* 2011; 32: 248-255.
2. Wark PA, Johnston SL, Bucchieri F, Powell R, Puddicombe S, Laza-Stanca V, Holgate ST, Davies DE. Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus. *J Exp Med* 2005; 201: 937-947.
3. Hackett TL, Singhera GK, Shaheen F, Hayden P, Jackson GR, Hegele RG, Van Eeden S, Bai TR, Dorscheid DR, Knight DA. Intrinsic phenotypic differences of asthmatic epithelium and its inflammatory responses to respiratory syncytial virus and air pollution. *Am J Respir Cell Mol Biol* 2011; 45: 1090-1100.
4. Heijink IH, Kies PM, Kauffman HF, Postma DS, van Oosterhout AJ, Vellenga E. Down-regulation of E-cadherin in human bronchial epithelial cells leads to epidermal growth factor receptor-dependent Th2 cell-promoting activity. *J Immunol* 2007; 178: 7678-7685.
5. Post S, Nawijn MC, Hackett TL, Baranowska M, Gras R, van Oosterhout AJ, Heijink IH. The composition of house dust mite is critical for mucosal barrier dysfunction and allergic sensitisation. *Thorax* 2012; 67: 488-495.
6. Post S, Nawijn MC, Jonker MR, Kliphuis N, van den Berge M, van Oosterhout AJ, Heijink IH. House dust mite-induced calcium signaling instigates epithelial barrier dysfunction and CCL20 production. *Allergy* 2013; 68: 1117-1125.
7. Wang H, Su Z, Schwarze J. Healthy but not RSV-infected lung epithelial cells profoundly inhibit T cell activation. *Thorax* 2009; 64: 283-290.

- 1 8. Heijink IH, Marcel KP, van Oosterhout AJ, Postma DS, Kauffman HF, Vellenga E. Der p, IL-
2 4, and TGF-beta cooperatively induce EGFR-dependent TARC expression in airway epithelium.
3 *Am J Respir Cell Mol Biol* 2007; 36: 351-359.
- 4 9. Borger P, Kauffman HF, Scholma J, Timmerman JA, Koeter GH. Human allogeneic CD2+
5 lymphocytes activate airway-derived epithelial cells to produce interleukin-6 and interleukin-8.
6 Possible role for the epithelium in chronic allograft rejection. *J Heart Lung Transplant* 2002; 21:
7 567-575.
- 8 10. Lordan JL, Bucchieri F, Richter A, Konstantinidis A, Holloway JW, Thornber M,
9 Puddicombe SM, Buchanan D, Wilson SJ, Djukanovic R, Holgate ST, Davies DE. Cooperative
10 effects of Th2 cytokines and allergen on normal and asthmatic bronchial epithelial cells. *J*
11 *Immunol* 2002; 169: 407-414.
- 12 11. Zhu L, Lee PK, Lee WM, Zhao Y, Yu D, Chen Y. Rhinovirus-induced major airway mucin
13 production involves a novel TLR3-EGFR-dependent pathway. *Am J Respir Cell Mol Biol* 2009;
14 40: 610-619.
- 15 12. Wood LG, Simpson JL, Wark PA, Powell H, Gibson PG. Characterization of innate immune
16 signalling receptors in virus-induced acute asthma. *Clin Exp Allergy* 2011; 41: 640-648.
- 17 13. Openshaw PJ. Immunity and immunopathology to respiratory syncytial virus. The mouse
18 model. *Am J Respir Crit Care Med* 1995; 152: S59-62.
- 19 14. Jackson DJ, Makrinioti H, Rana BM, Shamji BW, Trujillo-Torralbo MB, Footitt J, Jerico D,
20 Telcian AG, Nikonova A, Zhu J, Aniscenko J, Gogsadze L, Bakhsoliani E, Traub S, Dhariwal J,
21 Porter J, Hunt D, Hunt T, Hunt T, Stanciu LA, Khaitov M, Bartlett NW, Edwards MR, Kon OM,
22 Mallia P, Papadopoulos NG, Akdis CA, Westwick J, Edwards MJ, Cousins DJ, Walton RP,

- 1 Johnston SL. IL-33-dependent type 2 inflammation during rhinovirus-induced asthma
- 2 exacerbations in vivo. *Am J Respir Crit Care Med* 2014; 190: 1373-1382.

3

Figure legend

Figure 1. Primary bronchial epithelial cells (PBEs) inhibit α -CD3/ α -CD28-induced T cell proliferation and associated type-2 cytokine production, an effect that is attenuated by viral stimulation and in asthma. **A)** Representative CFSE dilution measurement. **B)** Proliferation of T cells cultured in direct contact with PBEs (Lonza) compared to T cells and PBEs separated in a transwell system ($n=5$). **C)** Proliferation of T cells cultured in conditioned medium (CM) of 24 hour-PBEC (Lonza) cultures ($n=5$). **D)** IFN- γ , IL-4, IL-5, IL-10 and IL-13 levels measured in cell-free supernatants by multiplex ELISA after T cell stimulation with α -CD3/ α -CD28-coupled beads for 4 days in the absence/presence of PBEs ((Lonza, $n=9-10$). **E)** Characteristics of the subjects from whom bronchial brushings were taken. Stable mild-moderate asthma patients were included based on the presence of allergy (assessed by skin test or Phadiatop) and bronchial hyperresponsiveness (assessed by PC₂₀ AMP<16mg/ml, PC₂₀ methacholine<8mg/ml or PC₂₀ histamine<8mg/ml), and the absence of other lung diseases and of any corticosteroid, long-acting β -agonist, and long-acting anticholinergic use for at least 4 weeks preceding the study. Medians (range) or number (%) are presented. FEV₁% pred =forced expiratory volume during the first second as percentage of predicted. **F)** Proliferation of T cells cultured with/without PBEs from controls or asthma patients. **G)** IL-13 levels in cell-free supernatants of the T cells co-cultured with/without PBEs from controls or asthma patients ($n=5$). **H)** Proliferation of T cells cultured alone, with PBEs (Lonza), or with PBEs (Lonza) that were pre-treated with 12.5 μ g/ml poly-(I:C) ($n=5$). **I)** Proliferation of T cells cultured with PBEs from asthma patients that were pre-treated with/without 12.5 μ g/ml poly-(I:C) ($n=3$). **J)** IL-13 levels in cell-free supernatants of the co-cultures described in H. Means \pm SEM are shown. *= $p<0.05$, **= $p<0.01$, and ***= $p<0.001$

- 1 as analyzed by 1-way ANOVA with Bonferroni's multiple comparison test in all graphs except
- 2 for panel D and I, where an unpaired and paired Student's t-test was used, respectively.